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The impact of melatonin and glutathione on the physiological characteristics of the hot pepper *Capsicum annuum* L. grown under cadmium pollution

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The experiment was conducted in pots within the plastic house of a nursery located in the Al-Muqdadiya district, situated to the north of Baqubah, the central city of Diyala governorate, which lies 90 km northeast of the capital, Baghdad. This study focused on *Capsicum annuum* L, specifically the Barbarian F1 class of Indian origin, during the autumn season. The objective of the experiment was to investigate the effects of melatonin and glutathione treatments on the physiological characteristics of *Capsicum annuum* L grown under conditions polluted with cadmium. The research followed a Randomized Complete Block Design (R.C.B.D.) with three replicates. Three treatments were applied: cadmium, added to the soil at concentrations of 0 and 15 mg.kg soil-1, and melatonin and glutathione, applied to the leaves at concentrations of 0, 50, and 100 mg.L^{-1}. The results revealed a significant decrease in fruit carbohydrate content and catalase enzyme activity when treated with cadmium at a concentration of 15 mg.kg soil-1. However, applying melatonin at concentrations of 50 and 100 mg.L^{-1} led to a significant increase in fruit carbohydrate and protein content, along with a notable rise in catalase and peroxidase enzyme activities. The concentration of 100 mg.L^{-1} demonstrated the highest mean values for these features. Moreover, the study demonstrated a significant increase in fruit carbohydrate and protein content, as well as catalase enzyme activity, when plants were treated with glutathione at concentrations of 50 and 100 mg.L^{-1}. The highest mean values for these features were observed when plants were treated with glutathione at a concentration of 100 mg.L^{-1}.

Keywords: Bioactive compounds, antioxidants, heavy metals, physiological characteristics, pollution, *Capsicum annuum* L.

INTRODUCTION

Heavy metals are elements with specific characteristics, characterized by an atomic number greater than 20 and a density larger than 5 g·cm^{-3} (Ali & Khan, 2018). Some of the most common polluting heavy metals include cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), and lead (Pb) (Varagiya *et al.*, 2022). These environmental pollutants are transmitted to humans through water, air, or food. In recent years, pollution from heavy elements has garnered significant attention from researchers due to its direct impact on plant growth. Heavy metal pollution hampers agricultural production, indirectly affecting human health as these elements are transferred through the food chain (Zhou *et al.*, 2020). Cadmium (Cd), a particularly damaging heavy metal, occurs naturally in the environment and is derived from agricultural and industrial sources. Cd enters the human body through contaminated food and water (Genchi *et al.*, 2020). Cd adversely affects various chemical, biological, morphological, physiological, and molecular processes crucial for plant development, leading to the loss of green color and stunted growth (Pérez-Chaca *et al.*, 2014).

Melatonin (N-acetyl-5-methoxytryptamine, MT) is a biologically active molecule with multiple functions, present in all animals and plants. Discovered in 1995 (Sharma & Zheng , 2019), melatonin plays a fundamental role in quelling the activity of free radicals, such as reactive nitrogen species (RNS) and reactive oxygen species (ROS). It enhances antioxidant capacity and guards against oxidative stress in cells, tissues, and organs. Essentially, melatonin acts as the first line of defense against potentially harmful conditions for living beings (Hernàndez-Ruiz & Arnao, 2019).

Glutathione (GSH), composed of three amino acids—glutamine (Glu), cysteine (Cys), and glycine (Gly)—is a water-soluble molecule with low molecular weight (Tan-b *et al.*, 2023). As one of the most non-enzyme antioxidants, glutathione plays multiple roles in plants, directly participating in preserving plant cells from damage caused by free radicals. This is achieved through three possible pathways: direct scavenging of ROS in the AsA-GSH cycle,

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removal of heavy metals by creating phytochelatin, or conjugation of catalyzed heavy metals by glutathione-S transferase (Cao *et al.,* 2017; Elkhatib *et al.,* 2021; Hasanuzzaman *et al.,* 2021).

Capsicum annuum L is a member of the Solanaceae family, which includes various vegetable crops, with tomato, eggplant, potato, and tobacco being among the most important (Karim *et al.,* 2021). Widely used across countries (Jarret *et al.,* 2019), hot pepper is valued for its delicious taste and high nutrient content (Colney *et al.,* 2018). Rich in essential nutrients like Potassium (K), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Sodium (Na), Iron (Fe), Manganese (Mn), Boron (B), Selenium (Se), Copper (Cu), and Zinc (Zn), the nutrient content in hot pepper depends on factors such as fruit type, growth stage, environmental influences, and agricultural practices (Zhang *et al.,* 2021). The present study aims to investigate the impact of different concentrations of melatonin and glutathione on the physiological characteristics of *Capsicum annuum* L. under conditions of cadmium pollution.

Materials: The experiment was conducted in pots within a plastic house located in Al-Muqdadiya district, north of Baqubah, the center of Diyala governorate, 90 km northeast of Baghdad. The experiment took place on October 6, 2022, using *Capsicum annuum* L, Barbarian F1 class, of Indian origin.

Experimental Design: A Randomized Complete Block Design (R.C.B.D.) was employed, including two cadmium concentrations ($CdCl_2$), 0 and 15 $mg \cdot kg^{-1}$ soil, and three concentrations of melatonin and glutathione (0, 50, 100 $mg \cdot L^{-1}$). There were a total of 18 treatments, as shown in Table 1. The treatments were replicated three times, resulting in a total of 54 experimental units. Each unit consisted of 5 pots, with one plant in each pot, bringing the total number of pots to 270.

Polluting the Experiment Soil: To introduce cadmium pollution in the study soil at a concentration of 15 mg.kg soil-1, a series of steps were undertaken. The soil was initially dried, softened, and sifted through a sieve with 5mm diameter holes. Subsequently, it was spread on the ground, which was covered with a thick nylon layer. Cadmium, dissolved in water, was then applied to the soil using a handheld sprayer, ensuring continuous stirring for thorough mixing. Following this, the soil was allowed to dry before being transferred to plastic pots. The pots had been prepared 30 days prior to transplanting hot pepper seedlings into them. Regular watering was maintained throughout the experiment.

Experimental Treatments: The hot pepper seedlings, at the 2-leaf stage, were obtained from a private nursery in Tarmiyah district, located 40 km north of Baghdad. Upon reaching the 3-leaf stage, the seedlings were individually transplanted into plastic pots. Melatonin was dissolved in 70% ethanol alcohol, and the volume was adjusted to 1 liter with distilled water. On the other hand, glutathione was directly dissolved in distilled water. Both melatonin and glutathione were applied foliarly in two stages, with a 3-day interval between the applications. The first stage occurred when the plants reached the 4-5-leaf stage, while the second stage was conducted during the flowering stage. Melatonin was initially sprayed on the plants, followed by the application of glutathione after three days. Additionally, 2-3 drops of liquid soap were added as a diffusing agent.

Table 1. Experimental Treatments.

No.	Treatments
T1	C0M0G0
T2	C0M0G1
T3	C0M0G2
T4	C0M1G0
T5	C0M1G1
T6	C0M1G2
T7	C0M2G0
T8	C0M2G1
T9	C0M2G2
T10	C1M0G0
T11	C1M0G1
T12	C1M0G2
T13	C1M1G0
T14	C1M1G1
T15	C1M1G2
T16	C1M2G0
T17	C1M2G1
T18	C1M2G2

The Studied Characteristics: From each experimental unit, three plants were selected randomly and they are marked for studying the following features:

The carbohydrate content of the fruit ($mg.g^{-1}$) was estimated following the methodology outlined by Dobiose et al. (1956). A Spectrophotometer device with a wavelength of 488nm was employed for the estimation process. The concentration of carbohydrates was determined using a standard glucose curve, calculated by the following equation:

Concentration of total dissolved carbohydrates($mg.100g^{-1}$) = Carbohydrates concentration from the standard curve X the final volume of the extract(ml.) X the number of dilutions/the sample weight

The fruit content of protein: This was estimated by using Kjeldahl Semi-micro device as appeared in (AOAC, 2008). The assessment of the raw protein was done by using the following equation:

Raw protein percentage (%) = [HCl amount (ml) X Standard (0.05) X 0.014x6.25x100] / [Sample weight x1000]

The catalase enzyme activity (absorption unit/minute/g fresh weight) in the leaves was assessed following the methodology outlined by Aebi (1984) using a spectrophotometer. Readings were taken at a wavelength of 420nm.

The peroxidase enzyme activity in leaves (absorption unit/minute/g fresh weight) was determined based on the procedure described by Müftügil (1985), utilizing a spectrophotometer with readings taken at a wavelength of 420nm.

***Statistical Analysis*:** The Statistical Analysis System (SAS) was employed to analyze the data, investigating the impact of various factors and their intersections with the studied features. This analysis was conducted based on the Randomized Complete Block Design (R.C.B.D) and was replicated three times for robustness, as indicated by the Analysis of Variance (ANOVA). To discern significant differences between means, the Least Significant Difference (LSD) test was utilized.

RESULTS

***The Fruits Content of Carbohydrates (mg.g^{-1})*:** Significant differences at the 5% probability level were observed in the means of the fruit carbohydrate content when treating plants with cadmium. The results in Table 2 show that the highest mean for this feature resulted from not adding cadmium, i.e., the control treatment with 0 mg.kg^{-1} soil, at 130.91 mg. In contrast, the lowest mean was observed with cadmium treatment at a concentration of 15 mg.kg^{-1} soil, yielding 111.51 mg and a decrease ratio of 14.81%.

The statistical analysis revealed significant differences at the 5% probability level in the means of the fruit carbohydrate content due to treating capsicum plants with melatonin concentrations of 0, 50, and 100 mg.L^{-1}. As presented in Table 2, the highest mean occurred when treating the plant with melatonin at a concentration of 100 mg.L^{-1}, reaching 145.45 mg. This represented an increase of 39.37% and 27.51% compared to the control treatment 0 mg.L^{-1} and the 50 mg.L^{-1} concentration, respectively. The control treatment recorded the lowest mean at 104.29 mg.

Additionally, significant differences at the 5% probability level were found in the means of the fruit carbohydrate content resulting from treating capsicum with glutathione at concentrations of 0, 50, and 100 mg.L^{-1}. The data in Table (2) indicated that the highest mean for this feature occurred when spraying the plant with glutathione at a concentration of 100 mg.L^{-1}, reaching 141.04 mg. This represented an increase of 42.10% and 14.34% compared to the control treatment 0 mg.L^{-1} and the 50 mg.L^{-1} concentration, respectively. The control treatment recorded the lowest mean at 99.25 mg, consistent with the results of Ghoname *et al.* (2010).

Furthermore, statistical analysis indicated significant differences at the 5% probability level in the means of this feature due to the binary interaction among cadmium, melatonin, and glutathione. As shown in Table (2), the best means for this character were obtained from the combination of cadmium 0 mg.L^{-1} + melatonin 100 mg.$^{L-}$1, cadmium 0 mg.kg^{-1} soil + glutathione 100 mg.L^{-1}, and melatonin 100 mg.L^{-1} + glutathione 100 mg.L^{-1}, resulting in 168.23 mg, 147.87 mg, and 177.11 mg, respectively.

Table (2) also indicated significant differences at the 5% probability level in the means of the fruit carbohydrate content due to the triple interaction among cadmium, melatonin, and glutathione. The best mean for this feature

Table 2. The Impact of Cadmium, Melatonin, and Glutathione and the Interactions among them on the Fruits Content of Carbohydrate (Mg.g^{-1}).

C	M	G			C+M	Average of C effect
		G0	G1	G2		
	M0	99.41	103.35	110.43	104.40	130.91
C1	M1	105.47	116.42	138.46	120.12	
	M2	143.94	166.03	194.73	168.23	
	M0	75.61	117.48	119.49	104.19	111.51
C2	M1	82.75	117.20	123.66	107.87	
	M2	88.32	119.64	159.49	122.49	
C+M+G		LSD: C+M+G =22.675*			LSD: C+M=22.706 NS	LSD: C= 7.558*
G + C						---
C1		116.27	128.60	147.87	LSD: C+G =22.706*	
C2		82.23	118.11	134.21		
G + M					Average of M effect	
M0		87.51	110.41	114.96	104.29	
M1		94.11	116.81	131.06	113.99	
M2		116.13	142.83	177.11	145.35	
LSD		LSD: M+G =20.121*			LSD: M =9.257*	
Average of G effect		99.25	123.35	141.04	---	
LSD value		LSD: G =9.257*				

Note: C= Cadmium, C1= 0 Mg.Kg^{-1} soil, C2= 15 Mg.Kg^{-1} soil, M= Melatonin, M0= 0 Mg.L^{-1}, M1= 50 Mg.L^{-1}, M2=100 Mg.L^{-1}, G= Glutathione, G0= 0 Mg.L^{-1}, G1= 50 Mg.L^{-1}, G2= 100 Mg.L^{-1}, *= Significant, N.S.= Non-Significant.

resulted from the combination of cadmium 0 mg.kg^{-1} soil + melatonin 100 mg.L^{-1}, reaching 194.73 mg. Conversely, the combination of cadmium 15 mg.kg^{-1} soil + melatonin 0 mg.L^{-1} + glutathione 100 mg.L^{-1} and the combination of melatonin 100 mg.L^{-1} + glutathione 0 mg.L^{-1} recorded the lowest mean 75.61 mg.

Determination of Fruits Content of Protein (%)**:** The statistical analysis results indicated no significant differences at the 5% probability level between the means of the protein content in hot pepper fruits resulting from treating with cadmium at a concentration of 15 mg/kg^{-1} soil. As shown in Table (3), the highest mean (1.57%) for this feature was obtained from the treatment without adding cadmium 0 mg/kg^{-1} soil, representing a 4.6% increase compared to the treatment with cadmium at 15 mg/kg^{-1} soil, which recorded the least mean at 1.50%.

Furthermore, the statistical analysis revealed significant differences at the 5% probability level between the means of the protein content in fruits resulting from melatonin treatment at concentrations of 50 and 100 mg.l^{-1}. Table (3) displayed the highest mean (1.77%) from the treatment with melatonin at 100 mg/l, with increases of 55.26% and 5.35% compared to the control treatment (0 mg/l) and the concentration of 50 mg.l^{-1}, respectively. The treatment without adding melatonin (0 mg/l) showed the least mean at 1.14%. These findings align with those reported by (El-Shieny *et al.,* 2022).

Significant differences at the 5% probability level were observed in the means of protein content in fruits resulting from treating the plant with glutathione at concentrations of 50 and 100 mg/l^{-1}. Table (3) indicated that the highest mean (1.77%) was obtained from treating the plants at a concentration of 100 mg.l^{-1}, with increases of 39.37% and 13.46% compared to concentrations of 0 mg/l and 50 mg.l^{-1}, respectively. The treatment without adding glutathione (0 mg.l^{-1}) recorded the least mean at 1.27%, consistent with the results reported by Ghoname *et al.*, 2010.

Additionally, there were significant differences at the 5% probability level between the means of protein content in hot pepper fruits due to the binary interaction among cadmium, melatonin, and glutathione. Table (3) showed that the highest means for this character were obtained from the combinations of cadmium 0 mg.kg^{-1} soil + melatonin 100 mg.l^{-1}, cadmium 15 mg.kg^{-1} soil + glutathione 100 mg.l^{-1}, and melatonin 100 mg.l^{-1} + glutathione 100 mg.l^{-1}, at 1.80%, 1.79%, and 1.98%, respectively. Conversely, the combinations of cadmium 15 mg.kg^{-1} soil + glutathione 0 mg.l^{-1} and melatonin 0 mg.l^{-1} + glutathione 0 mg.l^{-1} recorded the least means at 1.13%, 1.11%, and 0.87%, respectively. Results indicated significant differences at the 5% probability level due to the triple interaction between cadmium, melatonin, and glutathione. The highest mean (1.99%) resulted from the combination of cadmium 15 mg.kg^{-1} soil + melatonin 100 mg.l^{-1} + glutathione 100 mg.l^{-1}, while the combination of cadmium 15 mg.kg^{-1} soil + melatonin 0 mg.l $^{-1}$+ glutathione 0 mg.l^{-1} recorded the least mean at 0.65%.

The activity of catalase enzyme (Absorbency unit/min/g fresh weight)**:** Significant differences at the 5% probability level were observed between the means of catalase enzyme activity resulting from cadmium treatment, as depicted in

Table 3. The Impact of Cadmium, Melatonin, and Glutathione and the Interaction among them on the Fruits Content of Protein (%).

C mg.Kg^{-1} soil	M mg.L^{-1}	G mg.L^{-1}			C+M	Average of C effect
		G0	G1	G2		
	M0	1.08	1.09	1.31	1.16	1.57
C1	M1	1.53	1.75	1.96	1.74	
	M2	1.68	1.75	1.98	1.80	
	M0	0.65	1.31	1.43	1.13	1.50
C2	M1	1.31	1.59	1.96	1.62	
	M2	1.38	1.87	1.99	1.74	
C+M+G		LSD: C+M+G =1.30 NS			LSD: C+M=0.68 NS	LSD: C= 0.43 NS
G + C						---
C1		1.43	1.53	1.75	LSD: C+G =0.683 NS	
C2		1.11	1.59	1.79		
G + M					Average of M Effect	
M0		0.87	1.20	1.37	1.14	
M1		1.42	1.67	1.96	1.68	
M2		1.53	1.81	1.98	1.77	
value LSD		LSD: M+G =0.827*			LSD: M = 0.53*	
Average of G effect		1.27	1.56	1.77	---	
LSD value		LSD: G =0.53 NS				

Note: C= Cadmium, C1= 0 Mg.Kg^{-1} soil, C2= 15 Mg.Kg^{-1} soil, M= Melatonin, M0= 0 Mg.L^{-1}, M1= 50 Mg.L^{-1}, M2=100 Mg.L^{-1}, G= Glutathione, G0= 0 Mg.L^{-1}, G1= 50 Mg.L^{-1}, G2= 100 Mg.L^{-1}, *= Significant, N.S.= Non-Significant.

Table 4. The Impact of Cadmium, Melatonin, and Glutathione and the interactions among them on the Activity of Catalase Enzyme (Absorbency unit/min/g fresh weight).

$C\ mg.kg^{-1}$ Soil	M $mg.L^{-1}$	G $mg.L^{-1}$ G0	G1	G2	C+M	Average of C effect
	M0	0.05	0.06	0.09	0.07	0.09
C1	M1	0.08	0.08	0.09	0.08	
	M2	0.09	0.12	0.15	0.12	
	M0	0.01	0.01	0.07	0.03	0.06
C2	M1	0.07	0.07	0.08	0.07	
	M2	0.07	0.08	0.08	0.08	
C+M+G		LSD: C+M+G =0.009*			LSD: C+M=0.01 7*	LSD: C= 0.003*
G + C						---
C1		0.07	0.09	0.11	LSD: C+G =0.017NS	
C2		0.05	0.05	0.08		
G + M					Average of M Effect	
M0		0.03	0.04	0.08	0.05	
M1		0.07	0.07	0.08	0.08	
M2		0.08	0.10	0.11	0.10	
value LSD		LSD: M+G =0.008*			LSD: M = 0.003*	
Average of G effect		0.06	0.07	0.09	---	
LSD value		LSD: G =0.003*				

Note: C= Cadmium, C1= 0 $Mg.Kg^{-1}$ soil, C2= 15 $Mg.Kg^{-1}$ soil, M= Melatonin, M0= 0 $Mg.L^{-1}$, M1= 50 $Mg.L^{-1}$, M2=100 $Mg.L^{-1}$, G= Glutathione, G0= 0 $Mg.L^{-1}$, G1= 50 $Mg.L^{-1}$, G2= 100 $Mg.L^{-1}$, *= Significant, N.S.= Non-Significant.

Table 4. The results highlight a notable decrease in catalase activity when treated with a 15 $mg.kg^{-1}$ concentration of cadmium. This treatment recorded the lowest mean at 0.06 units of absorbency per minute, reflecting a 33.33% decrease compared to the control treatment with 0 mg/kg soil, which registered the highest mean at 0.09 units of absorbency per minute.

Furthermore, the results indicate significant differences at the 5% probability level in catalase activity resulting from melatonin treatment. Table 4 reveals that the highest mean was obtained at a concentration of 100 $mg.L^{-1}$, recording a mean of 0.10 and an increase of 100% and 25% compared to the control treatment (0 $mg.L^{-1}$) and the 50 $mg.L^{-1}$ concentration, respectively. In contrast, the control treatment recorded the lowest mean at 0.05 units of absorbency per minute. These findings align with previous research by Ali *et al.*, 2020.

The study also unveils significant differences at the 5% probability level in catalase activity resulting from glutathione treatment at concentrations of 0, 50, and 100 $mg.L^{-1}$. The highest mean, 0.09 units of absorbency per minute, was observed at a concentration of 100 $mg.L^{-1}$, showing a 50% and 28.57% increase compared to the control treatment (0 $mg.L^{-1}$) and the 50 $mg.L^{-1}$ concentration, respectively. Conversely, the control treatment recorded the lowest mean at 0.06 units of absorbency per minute.

Moreover, significant differences at the 5% probability level were noted in the catalase activity feature due to the binary interaction among cadmium, melatonin, and glutathione. Table (4) reveals that the most favorable means for this feature resulted from combinations such as cadmium 0 $mg.kg^{-1}$ soil + melatonin 100 $mg.L^{-1}$, cadmium 0 $mg.kg^{-1}$ soil + glutathione 100 $mg.L^{-1}$, and melatonin 100 $mg.L^{-1}$ + glutathione 100 $mg.L^{-1}$. These means were 0.12, 0.11, and 0.11 units of absorbency per minute, respectively.

Additionally, Table (4) explains significant differences at the 5% probability level in the catalase activity of capsicum due to the triple interaction among cadmium, melatonin, and glutathione. The best mean for this feature was obtained through the combination of cadmium 0 $mg.kg^{-1}$ soil + melatonin 100 $mg.L^{-1}$ + glutathione 100 $mg.L^{-1}$, reaching 0.15 units of absorbency. Conversely, the combination of cadmium 15 $mg.kg^{-1}$ soil + melatonin 0 $mg.L^{-1}$ + glutathione 0 $mg.L^{-1}$ recorded the lowest mean at 0.01 unit of absorbency per minute.

***The Activity of Peroxidase Enzyme (Absorbency unit /min/g fresh weight)*:** The statistical analysis results indicate no significant differences at the 5% probability level between the means of peroxidase enzyme activity when treating pepper plants with cadmium at a concentration of 15 mg/kg soil. Table 5 illustrates that the highest mean for this trait was 0.39 units of absorbency per minute. This mean was obtained from the treatment with 15 $mg.kg^{-1}$ soil, showing an increase to 8.33 units of absorbency per minute compared to the non-cadmium treatment (0 $mg.kg^{-1}$ soil), which recorded the lowest mean at 0.36 units of absorbency per minute.

Table 5 also reveals significant differences at the 5% probability level in peroxidase activity when treating plants with melatonin at concentrations of 50 and 100 $mg.L^{-1}$. The highest mean for this trait was observed with the treatment of

Table 5. The Impact of Cadmium, Melatonin, and Glutathione and the interactions among them on the Activity of Peroxidase Enzyme (Absorpency unit/min/g fresh weight).

C $Mg.Kg^{-1}$ soil	M $Mg.L^{-1}$	G $Mg.L^{-1}$			C+M	Average of C effect
		G0	G1	G2		
	M0	0.19	0.12	0.19	0.44	0.36
C1	M1	0.19	0.30	0.41	0.30	
	M2	0.22	0.34	0.43	0.33	
	M0	0.22	0.23	0.28	0.25	0.39
C2	M1	0.20	0.25	0.31	0.26	
	M2	0.57	0.61	0.85	0.68	
C+M+G		LSD: C+M+G =0.390 *			LSD: C+M=0.266*	LSD: C= 0.130NS
G + C						---
C1		0.47	0.25	0.34	LSD: C+G =0.266 NS	
C2		0.33	0.36	0.48		
G + M					Average of M effect	
M0		0.20	0.28	0.36	0.28	
M1		0.61	0.17	0.24	0.34	
M2		0.39	0.47	0.64	0.50	
LSD		LSD: M+G =0.328*			LSD: M = 0.159*	
Average of G effect		0.40	0.31	0.41	---	
LSD value		LSD: G = 0.159 NS				

Note: C= Cadmium, C1= 0 $Mg.Kg^{-1}$ soil, C2= 15 $Mg.Kg^{-1}$ soil, M= Melatonin, M0= 0 $Mg.L^{-1}$, M1= 50 $Mg.L^{-1}$, M2=100 $Mg.L^{-1}$, G= Glutathione, G0= 0 $Mg.L^{-1}$, G1= 50 $Mg.L^{-1}$, G2= 100 $Mg.L^{-1}$, *= Significant, N.S.= Non-Significant.

melatonin at 100 $mg.L^{-1}$, reaching 0.50 units of absorbency per minute. This represented an increase of 78.56% and 47.05% compared to the control treatment (0 $mg.L^{-1}$) and the 50 $mg.L^{-1}$ concentration, respectively. The lowest mean was recorded for the non-melatonin treatment (0 $mg.L^{-1}$), with a value of 0.28 units of absorbency per minute.

Significant differences were noted at the 5% probability level in peroxidase activity for capsicum treated with glutathione at concentrations of 50 $mg.L^{-1}$ and 100 $mg.L^{-1}$. The highest mean for this feature, 0.41 units of absorbency, resulted from treating plants with 100 mg/L glutathione, showing a 2.5% increase compared to the control (0 $mg.L^{-1}$) and a 32.25% increase compared to the 50 mg/L concentration. The lowest mean, 0.31 units of absorbency per minute, was observed with the treatment of 50 $mg.L^{-1}$ glutathione.

The results in Table 5 indicate significant differences at the 5% probability level in peroxidase activity for capsicum due to interactions between cadmium, melatonin, and glutathione. The highest means were observed in combinations such as cadmium 15 $mg.kg^{-1}$soil + melatonin 100 $mg.L^{-1}$, cadmium 15 $mg.kg^{-1}$ soil + glutathione 100 $mg.L^{-1}$, and melatonin 100 $mg.L^{-1}$ + glutathione 100 $mg.L^{-1}$, with values of 0.68, 0.48, and 0.64 units of absorbency per minute, respectively. The lowest means were recorded in combinations like cadmium 15 $mg.kg^{-1}$ soil + melatonin 0 $mg.L^{-1}$, cadmium 0 $mg.kg^{-1}$ soil + glutathione 50 $mg.L^{-1}$, and melatonin 50 $mg.L^{-1}$ + glutathione 50 $mg.L^{-1}$, with values of 0.25, 0.25, and 0.17 units of absorbency per minute, respectively.

Moreover, significant differences at the 5% probability level were found in peroxidase activity for capsicum due to triple interactions among cadmium, melatonin, and glutathione. The highest mean was recorded for the combination of cadmium 15 $mg.kg^{-1}$ soil + melatonin 100 $mg.L^{-1}$ + glutathione 100 $mg.L^{-1}$, reaching 0.85 units of absorbency per minute. The lowest mean was observed in the combination of cadmium 0 $mg.kg^{-1}$ soil + melatonin 0 $mg.L^{-1}$ + glutathione 50 $mg.L^{-1}$, with a value of 0.12 units of absorbency per minute.

The research results suggest that the interaction between melatonin and glutathione, especially at higher concentrations, plays a crucial role in enhancing the activity of antioxidant enzymes. This enhancement serves as a defense mechanism against the oxidative stress induced by cadmium poisoning at a concentration of 15 $mg.kg^{-1}$ soil.

DISCUSSION

Pollution with heavy metals represents one of the most significant stresses that plants encounter throughout their various growth stages. Our findings corroborate the results of a study by Ghoname *et al*. (2010). The decrease in fruit carbohydrate content following cadmium treatment is attributed to abiotic pressures, such as heavy metals activating starch synthesis for fats. Carbon, typically used for constructing carbohydrates and proteins, is diverted toward biosynthesizing fats (Chen *et al*., 2017). Another contributing factor to this decrease is the reduction in CO2 fixation in plants exposed to pollution with cadmium (Krantev *et al.*, 2008).

Regarding the effect of melatonin on fruit carbohydrate content, the observed increment is linked to melatonin's role

in enhancing photosynthetic efficiency. This, in turn, aids in producing the carbohydrates necessary for sustaining growth by mitigating the impact of external stress on the plant (Iqbal *et al.,* 2021). As for the influence of glutathione on carbohydrate content, the results indicate an increase, likely attributed to the amino acids it contains, enhancing chlorophyll biosynthesis or mitigating its decomposition. Consequently, this leads to an increase in photosynthesis and crop productivity with stored carbohydrates (Dawood *et al.,* 2020).

Regarding protein content in fruits, the addition of melatonin is associated with an increase due to its role in mitigating protein decomposition and enhancing the synthesis of new proteins (Mushtaq *et al.,* 2022). Conversely, the addition of outer glutathione results in increased protein content, attributed to the synthesis of stress-related proteins through the promotion of antioxidant enzyme production linked to the cell wall. This, in turn, enhances the activity and stability of cell proteins (Keutgen *et al.,* 2007).

It was observed that cadmium enhances the production of free radical types of reactive oxygen species (ROS) in plants. These radicals interact with fats, proteins, nucleic acids, and other substances, leading to fat peroxidation, membrane destruction, and enzyme inactivation. Consequently, this affects plant performance and vitality (Ahmed *et al.,* 2010). Conversely, treatment with melatonin leads to an increase in catalase activity due to its role in enhancing antioxidant enzyme activity, thereby reducing oxidative stress resulting from cadmium accumulation in the plant, our research results demonstrate that melatonin treatment increases peroxidase enzyme activity, as melatonin acts as an antioxidant, motivating the antioxidant system to reduce ROS levels under stress induced by heavy metal pollution (Siddiqui *et al.,* 2020).

Conclusions: From the results of this study, it can be concluded that cadmium negatively impacts the physiological features of the plant. Both melatonin and glutathione play a role in mitigating this impact through various mechanisms, including the increased activity of antioxidant enzymes such as catalase and peroxidase. These enzymes serve as a defense mechanism against the oxidative stress induced by the presence of cadmium at a concentration of 15 $mg.kg^{-1}$ soil.

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